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Current Opinion in Virology

Host genes and influenza pathogenesis in humans: an emerging paradigm

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The emergence of the pandemic influenza virus A(H1N1)pdm09 in 2009 and avian influenza virus A(H7N9) in 2013 provided unique opportunities for assessing genetic predispositions to severe disease because many patients did not have any underlying risk factor or neutralizing antibody against these agents, in contrast to seasonal influenza viruses. High-throughput screening platforms and large human or animal databases from international collaborations allow rapid selection of potential candidate genes for confirmatory functional studies. In the last 2 years, at least seven new human susceptibility genes have been identified in genetic association studies. Integration of knowledge from genetic and phenotypic studies is essential to identify important gene targets for treatment and prevention of influenza virus infection.

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Current Opinion in Virology 2015, **14**:7–15

This review comes from a themed issue on **Engineering for viral resistance**

Edited by **Albrecht von Brunn**

For a complete overview see the Issue and the Editorial

Available online 14th June 2015

http://dx.doi.org/10.1016/j.coviro.2015.04.010

Introduction

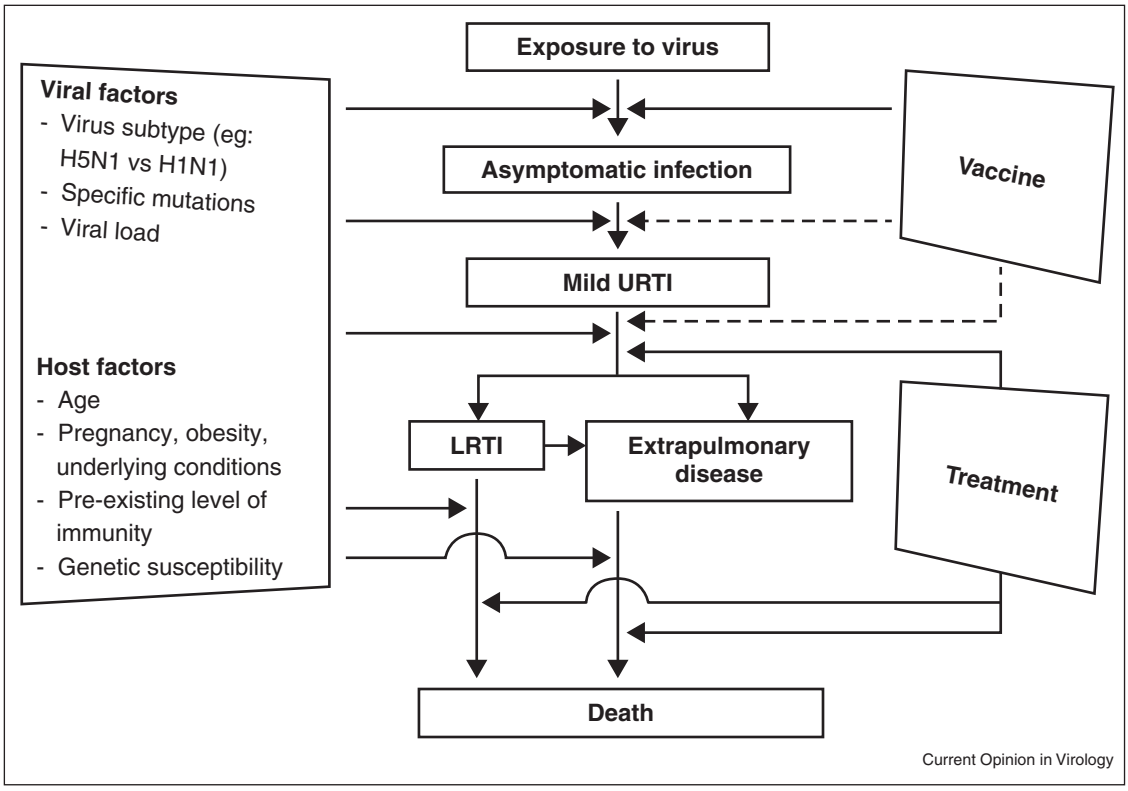
Influenza virus is one of the most common seasonal respiratory viruses affecting humans, leading to 250 000–500 000 deaths every year [1]. The 2009 pandemic A(H1N1) virus (A[H1N1]pdm09 virus) was estimated to cause 201 000 respiratory deaths in the first 12 months, with most deaths occurring in patients aged <65 years [2,3•]. The avian influenza viruses A(H5N1), A(H7N9), A(H10N8), A(H5N6), together with the severe acute respiratory syndrome and Middle East respiratory syndrome coronaviruses, are the most virulent respiratory viruses affecting humans [4•,5–7]. Most patients with influenza virus infection develop mild upper respiratory tract infection. Life-threatening complications include severe viral pneumonia or secondary bacterial pneumonia, acute respiratory distress syndrome, pulmonary embolism, myocarditis, encephalopathy, Reye's syndrome, hemophagocytic syndrome, multiorgan dysfunction and exacerbation of underlying chronic cardiovascular and respiratory diseases [3•,4•]. Currently available antivirals and vaccines have limited efficacy in the treatment and prevention of influenza virus infection [8]. Understanding the pathogenesis of influenza virus infection in human is important in designing novel strategies to improve the management of influenza.

To cause infection, influenza virus needs to evade the host immunity, enter and replicate inside host cells, and disseminate to other cells or organs. Host damage can be a result of direct virus-induced damage, immune-mediated damage and/or secondary bacterial infection [9–11]. Virologists have extensively studied the role of viral components in the viral life cycle and in the pathogenesis of influenza virus infection in humans [12]. Specific amino acid changes in the viral proteins have been associated with increased disease severity in humans or adaptation of avian influenza viruses in humans [13]. Clinical risk factors for severe influenza have been well described (Figure 1) [3•]. However, specific human genes regulating the influenza virus life cycle or virus-induced inflammatory responses are less well described.

Traditionally, the study of host genes depends on prior knowledge of a candidate gene. The importance of the candidate gene is verified using single gene knockout/knockdown or gain-of-function studies with reverse genetics *in vitro* or *in vivo*. For example, Chinese and Japanese patients with influenza-associated encephalopathy (IAE) were found to have elevated (C16:0 + C18:1)/C2 acylcarnitines ratios. Carnitine palmitoyltransferase 2 (CPT2) variants F352C and V368I were found to be overrepresented in these IAE patients when compared with the general population. These mutations render this enzyme susceptible to inactivation at high temperature occurring in febrile patients [14,15].

High-throughput screening platforms have allowed researchers to systematically screen a large number of genes associated with influenza virus infection *in vitro*, in animals or in humans. The availability of deep sequencing data of the human genome allows researchers to compare genetic variations between influenza patients and the general population [16•]. These technological

Figure 1



Natural course of influenza virus infection. LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection.

advances, combined with *in vitro* or bioinformatics analysis, have revealed several genes associated with severe influenza (Figure 2 and Table 1). In this review, we focus on specific host genes that have been shown to be directly related to the pathogenesis of human influenza identified or with updated knowledge in the past 2 years.

Antimicrobial molecules in the airway

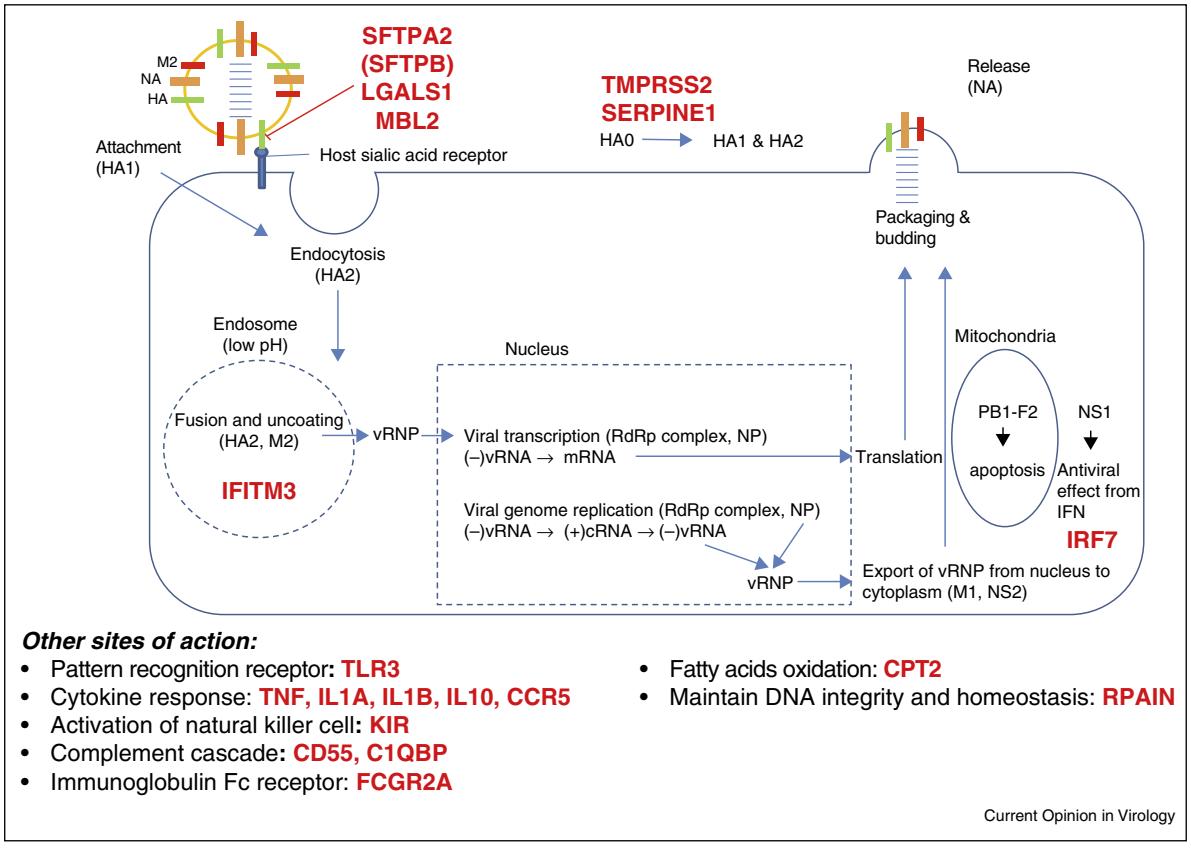
Four types of surfactant proteins are found in human pulmonary surfactant. Both surfactant protein A2 (SFTPA2) and surfactant protein D (SFTPD) are collectins and exhibit antiviral activity against influenza virus [17]. *SFTPA2* variants (rs1965708-C, rs1059046-A, and haplotype 1A[0]) have been associated with more severe respiratory deterioration [18]. Surfactant protein B (SFTPB) is a hydrophobic protein responsible for the structural integrity of the pulmonary alveoli [19]. In a cohort of 84 Chinese patients with severe and mild A(H1N1)pdm09 infection who were matched for age, sex and underlying conditions, the SNP rs1130866-C was significantly associated with severe disease. The association between rs1130866-C and severe disease was verified in a second cohort patients with A(H1N1)pdm09 infection, in which multivariate analysis was performed to adjust for potential confounding factors [20••]. The SNP rs1130866-C has been associated with glycosylation at the amino acid residue 129 of SFTPB, which reduces secretion of SFTPB [21]. It remains to be determined whether SFTPB has direct antiviral activity against influenza virus. Surfactant protein C (SFTPC) is a lipoprotein with antiviral activity against respiratory syncytial virus [22], but its antiviral activity against influenza virus or its genetic variants associated with severe influenza has not yet been reported.

Lectin, galactoside-binding, soluble, 1 (LGALS1), also known as galectin 1, can bind to influenza viruses and inhibit viral replication [23]. Carriage of *LGALS1* rs4820294/rs2899292 haplotype GG was associated with protection from A(H7N9) virus infection in humans. Furthermore, rs4820294/rs2899292 haplotype GG was correlated with higher levels of *LGALS1* mRNA and protein expression in lymphoblast cell lines [24•]. Therefore, the differential *LGALS1* expression may contribute to the distinct susceptibility of some individuals to human A(H7N9) influenza.

The interferon pathway

Upon influenza virus infection, type I interferons are produced by host cells to limit viral replication. The

Figure 2



Host genetic determinants of influenza virus disease severity identified in humans. Host genes that have been associated with severe influenza are highlighted in red.

function of type I interferons is mediated via the expression of many interferon stimulated genes (ISGs). Several ISGs are especially important in the pathogenesis of influenza virus infection. Interferon-induced transmembrane proteins (IFITM), including IFITM1, IFITM2 and IFITM3, were identified to have direct antiviral activity against influenza virus in a genomewide siRNA screen [25]. A human long noncoding RNA, which reduces *IFITM3* gene expression, enhanced influenza virus replication in a transgenic mouse model [26•]. IFITM3, which is located in endosome, inhibits viral replication by blocking the fusion of the viral and the host membrane [27,28]. Interestingly, amphotericin B, an antifungal, was found to increase viral replication by interfering with the blockage of membrane fusion by IFITM3 [29]. The C/C genotype of rs12252 is associated with a 21-amino-acid truncation at the N-terminal of the IFITM3 protein, which alters the localization of the IFITM3 from endosomal compartment to the cell periphery [30]. Several studies have looked into *IFITM3* SNP rs12252 in the susceptibility to severe influenza in humans. The genotype rs12252-C was over-represented in Caucasian and Chinese patients with severe A(H1N1)pdm09 virus infection [31••,32]. In patients with A(H7N9) infection, those carrying rs12252-C/C genotype had higher mortality [33]. However, the importance of rs12252-C allele in severe influenza has been challenged. Firstly, in a study using pseudotyped influenza A viruses, transfection of A549 cells with plasmids expressing the truncated form of *IFITM3* could reduce viral replication similarly to those with plasmids carrying the full length *IFITM3* [34]. Secondly, in a study comparing 34 patients with severe A(H1N1)pdm09 virus infection and >5000 controls, no significant difference in the rs12252 genotype frequency was found [35].

Interferon regulatory factor 7 (IRF7) is a transcription factor regulating the expression of type I interferons [36]. Influenza A virus replicates to high titers in Madin-Darby canine kidney cells with knockdown of the *IRF7* gene [37]. Murine tracheal epithelial cells deficient in IRF7 have impaired expression of interferons after influenza virus infection [38]. In a study comparing the genetic variants in 534 healthy individuals, *IRF7* SNP rs12805435

Table 1

Host genes associated with severe influenza virus in humans

Gene	Human studies			Animal studies	Phenotypic studies in cell lines *in vitro*	References
	Genetic association study from large patient cohorts	Genetic variants identified from small patient cohorts with severe disease	Comparison of different genetic variants from human cells		Confirmation of functional significance of identified genetic variant	
IFITM3	+[a,b]	+	–	+	+	[25,26•,27,28,29,30,32,33,35,79]
IRF7	–	+	–	+	+	[38,39,40••]
SERPINE1	–	–	+	+	+	[47••]
TMRPSS2	+	–	–	+	+	[42–46,80]
LGALS1	+	–	–	+	+	[23,24•]
MBL2	+[b]	–	–	–	+[c]	[18,54,81]
SFTPA2	+	–	–	–	+	[18]
SFTPB	+[a]	–	–	–	–	[20••]
CD55	+[a]	–	–	–	+	[82]
C1QBP	+	–	–	–	–	[58]
FCGR2A	+[b]	–	–	–	–	[58,59]
CPT2	+	+	–	–	+	[14,15,83]
TNF	+[b]	–	–	+	+	[48–50,52–54,84]
IL-1A, IL-1B	+	–	–	–	+	[56,57]
TLR3	+	+	–	+	+	[63–65]
KIR	+[a]	–	–	–	–	[85,86]
CCR5	+[b]	+	–	–	–	[87–90]
RPAIN	+	–	–	–	–	[58]

Abbreviations: C1QBP, complement component 1 Q subcomponent-binding protein; CCR5, chemokine (C-C motif) receptor 5; CD55, CD55 molecule, decay accelerating factor for complement; CPT2, carnitine palmitoyltransferase 2; FCGR2A, Fc fragment of IgG, low affinity IIa, receptor; IFITM3, interferon-induced transmembrane protein 3; IL, interleukin; IRF7, interferon regulatory factor 7; ISG, interferon stimulated genes; KIR, killer-cell immunoglobulin-like receptors; LGALS1, lectin, galactoside-binding, soluble, 1; MBL2, mannose binding lectin 2; RPAIN, RPA interacting protein; SFTPA2, surfactant protein A2; SFTPB, surfactant protein B; TLR3, toll like receptor 3; TMPRSS2, transmembrane protease, serine 2; TNF, tumor necrosis factor.

[a] Significant association demonstrated in more than one genetic association study or in more than one cohort of patients.

[b] At least one study showing no statistical significance between severe cases and controls.

[c] Does not inhibit A(H1N1)pdm09 because this virus lacks a paucity of glycan.

was found to be associated with the induction of antiviral genes in dendritic cells in response to influenza virus infection [39]. In a 7-year-old girl without known immunodeficiency who suffered from severe influenza virus infection, *IRF7* mutation was identified using whole exome sequencing [40••]. The induction of type I and type III interferon genes in dendritic cells and pulmonary epithelial cells were found to be impaired in this girl.

Proteolytic activation of viral hemagglutinin

Post-translational cleavage of influenza virus hemagglutinin by host protease is a prerequisite for the virus-host membrane fusion and thereby, for virus infectivity, tissue tropism and virus pathogenicity [41]. Transmembrane protease, serine 2 (TMPRSS2), a type II transmembrane serine protease, cleaves and activates the viral hemagglutinin during influenza virus infection [42]. Three independent studies assessed the role of TMPRSS2 in influenza-infected mice. *TMPRSS2* knockout mice were resistant to influenza A(H7N9) and A(H1N1) virus infection, but there were discrepant results regarding susceptibility to A(H3N2) virus infection [43–45]. By integration of a pilot genomewide association study and the lung expression quantitative trait loci (eQTL) dataset, a *TMPRSS2* intronic SNP rs2070788 was prioritized for further studies [46]. The genetic predisposition of rs2070788 to severe A(H1N1)pdm09 was validated in 409 A(H1N1)pdm09 patients including 162 severe cases and 247 mild controls. In the functional study, a regulatory SNP rs383510 in high linkage disequilibrium with rs2070788 was uncovered as the causal variant underlying the genetic association. Genetic predispositions of rs2070788 and rs383510 to severe influenza were also validated in an A(H7N9) patient cohort.

SERPINE1, an ISG, encodes plasminogen activator inhibitor 1 (PAI-1). A549 cells over-expressing *SERPINE1* inhibited the spread of influenza A virus when compared to cells without *SERPINE1* overexpression [47••]. PAI-1 inhibits the extracellular cleavage of hemagglutinin from HA0 into HA1 and HA2. Serpine1 knockout mice had higher viral titers and more severe disease than wild type mice. In human fibroblast cell lines derived from patients carrying rs6092-A allele which results in intracellular

retention of PAI-1, influenza virus replication was enhanced when compared with infection in cell lines deriving from patients carrying rs6092-T/T genotype.

Pro-inflammatory and anti-inflammatory cytokines

Cytokines are important in the defense against influenza virus infection. However, excessive cytokine response is associated with severe influenza [9,32]. Tumor necrosis factor-α (TNF-α) has been considered a proinflammatory cytokine, and treatment with anti-TNF-α improved the survival of A(H1N1)-infected mice [48]. However, *TNF*-knockout mice had more severe disease [49]. Furthermore, the soluble form of TNF-α has been shown to be required for the control of $CD8^+$ T cell response [50], which suggests that TNF-α is required for the control of infection. A network based approach combining mouse, human and *in vitro* data showed that the *TNF* pathway is important in influenza virus infection [51•]. *TNF*-238A and *TNF*-308G alleles have been associated with severe influenza in studies comparing A(H1N1)pdm09 patients and healthy controls [52,53]. However, these associations were not found in another study comparing fatal cases and the general population [54].

Interleukin-10 (IL-10) is persistently elevated in patients with severe influenza virus infection [9]. *IL-10* knockout mice were shown to have more rapid viral clearance and improved survival [55]. Blockage of IL-1β could ameliorate inflammation of influenza-virus infected human pulmonary endothelial cells and lung fibroblasts [56]. *IL-10*-592C, *IL-10*-1082A allele and *IL-10*-1082 A/A genotype have been associated with severe disease [53]. In another study involving 167 patients with A(H1N1)pdm09 virus infection and 192 healthy controls, rs17561 of *IL1A* and rs1143627 of *IL1B* gene were associated with susceptibility to infection [57]. However, the associations of SNPs in *IL-10*, *IL-1A* and *IL-1B* genes with influenza virus disease severity were based on single cohorts, and further studies are necessary to confirm the significance of these polymorphisms in influenza.

Immunoglobulin Fc receptor

The interaction between the host antibody Fc region and Fc receptors is important in the immune defense against influenza virus infection. Fc fragment of IgG, low affinity IIa, receptor (*FCGR2A*) gene encodes Fcγ receptor IIA (FcγRIIA), which binds immune complexes. The association between *FCGR2A* polymorphism and severe A(H1N1)pdm09 virus infection was first identified in a European cohort [58], although such association was not found in a Chinese cohort [59]. FcγRIIA signaling was found to be important in immune complex-mediated platelet activation during influenza virus infection [60]. Interestingly, in recent studies of broadly neutralizing antibodies against influenza viruses, the ability to form immune complex by the interaction between Fc region of the antibody and Fcγ receptors may mediate antibody-dependent cellular cytotoxicity, which is important for *in vivo* efficacy of the antibody [61,62].

Pattern recognition receptor

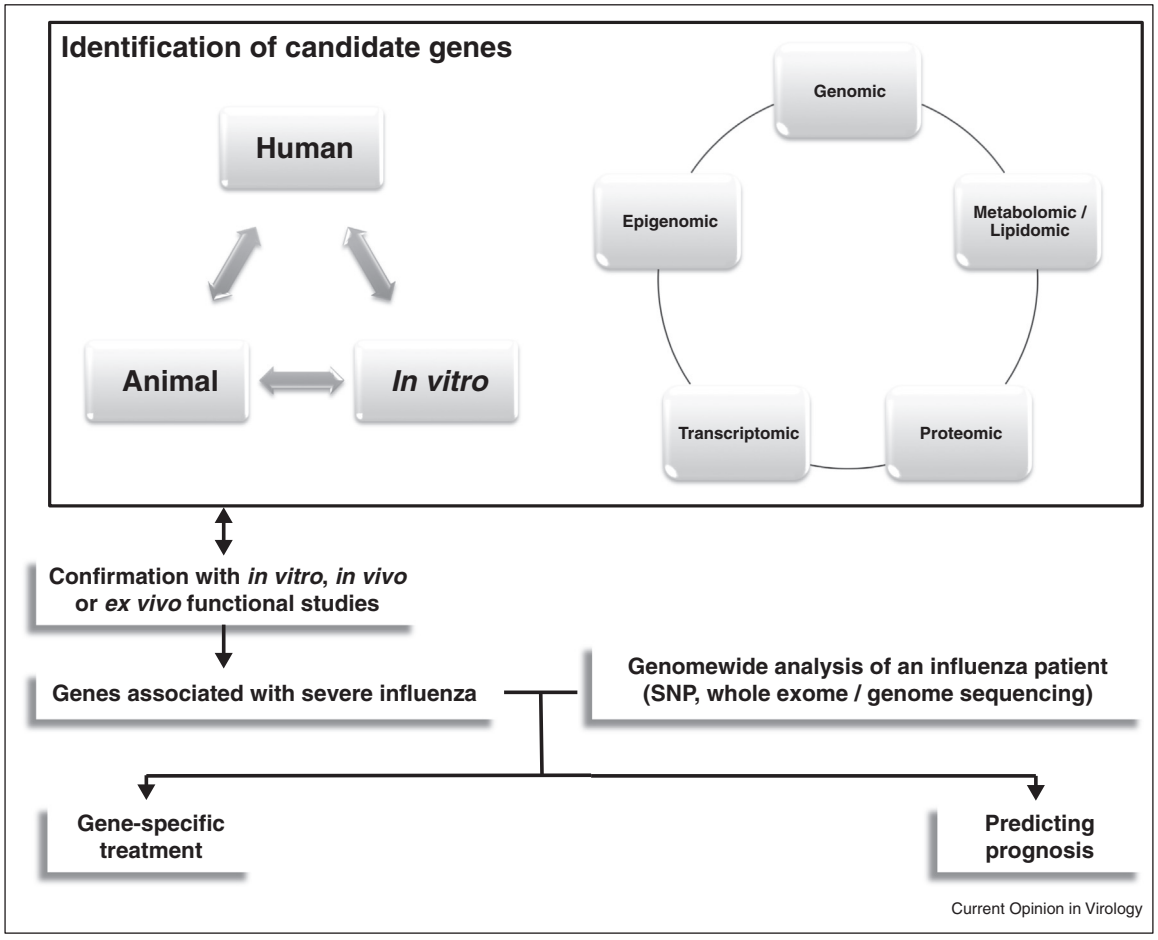
TLR3 is a major sensor of viral double stranded RNA. F303S mutation of TLR3 was found to be associated with influenza-associated encephalopathy [63], and SNP rs5743313-C/T genotype was associated with A(H1N1)pdm09 patients with pneumonia [64]. The importance of TLR3 has been subsequently confirmed in a knockout mice study [65].

The future on the study of host genes in humans

This review has summarized host genes that have been shown to be important for the pathogenesis of influenza in humans. With high-throughput assays, a large number of genes or genetic variants were found to be associated with viral replication or disease severity in animals or in humans. The current challenge is how we can select specific host genes that are important for the treatment or prevention of influenza virus infection. Discrepancies in the results from similar studies also raise the suspicion whether certain genes are indeed important. For example, out of 925 host factors which affect influenza virus replication that were identified during *in vitro* genomewide siRNA knockdown screening, only 69 genes were present in at least two of these screens [66]. In human genetic association studies, many genetic variants identified to be associated with disease severity in one study could not be reproduced in other studies (Table 1).

Several strategies can be used to improve our ability to find genes that are relevant to influenza pathogenesis in humans. In human genetic association studies, it is important to limit confounding factors as much as possible. For example, the cases and controls should be matched for age, sex, ethnic group and comorbidities, and multivariate analysis can be utilized to assess whether the genetic variant is an independent risk factor. The identified genetic variants should be validated in other independent cohorts. The functional significance of these genetic variants can be refined by eQTL analysis [67]. Pathway analysis helps to identify pathways that may not be apparent when analyzing individual genes. Interactome screens, which identify host genes that interact with viral proteins, may increase the likelihood of identifying genes that are potential host targets for treatment [68]. The establishment of genetically diverse mice population has allowed the identification of susceptibility genes by correlating genetic variations to disease phenotypes [69•]. In addition to genetic data, host response to influenza has also been assessed using transcriptomic, proteomic, metabolomic and lipidomic analysis of cell cultures, animals or humans [70•,71]. These data reflect biological regulation

Figure 3



Pathways of identifying and characterizing susceptibility genes in human and potential clinical application.

of host factors at different levels. An integration of these 'omics' data will improve the accuracy of identifying the susceptibility genes [72••].

Although studies have identified several genetic polymorphic genes that predispose to severe influenza, it is possible that these susceptible individuals may have very different susceptibility gene combinations. In the future, it is possible that whole genome sequencing will allow more accurate identification of susceptibility gene combinations in any particular individual (Figure 3).

In addition to comparing severe and mild influenza cases, another approach to identify host susceptibility genes is to examine patients who are resistant to influenza virus infection. In a study of a patient with a congenital disorder of glycosylation but without serological evidence of influenza virus infection, virus production was only found in 1 of 3 macrophage cultures derived from this patient [73]. The authors of this study postulated that the lack of glycosylation of viral surface proteins may affect virus production.

Conclusion

Understanding the role of host factors in virus infections have led to host-targeted antivirals. DAS181 removes sialic-acid containing receptors from respiratory epithelial cells and prevents virus attachment to host cells. In a phase II clinical trial, DAS181 has been shown to reduce nasal or pharyngeal viral load in influenza patients [74]. Antivirals and anti-inflammatory agents targeting host factors and vaccine strategies improving host response have shown promise in animal models. For example, sphingosine-1-phosphate agonist, protectin D1 and anti-leptin antibody have been shown to protect mice against lethal influenza virus infection [70•,75,76]. Imiquimod, a TLR7 agonist, has been shown to expedite and augment antibody response after influenza virus vaccine in both animal and human studies [77,78]. An integration of *in vitro*, animal and clinical data will allow researchers

to search for other host targets for treatment and prevention of influenza virus infection.

Acknowledgements

Our studies presented in this review are supported by the Health and Medical Research Fund of the Food and Health Bureau of the Hong Kong SAR Government [Ref. No. 13120842, 12111412, RRG-05 and HKM-15-M03], National Key Program for Infectious Diseases of China (Ref. No. 2012ZX10004210), the Providence Foundation Limited in memory of the late Dr Lui Hac Minh, and a donation from Larry Chi-Kin Yung.

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- •• of outstanding interest

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